Ultraviolet Absorption Spectroscopy

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Most fat and oil chemists are somewhat aware of the utility of ultraviolet absorption spectroscopy although they may not be cognizant of the wide scope of its present-day applications and are entirely unaware of its still vast potentialities. The main objective of this discussion is to show how ultraviolet absorption spectroscopy is being applied to the solution of a variety of problems in fat and oil chemistry. But before citing specific examples of its successful use, it is probably desirable to define the term "ultraviolet absorption spectroscopy" to attempt to create a clear picture of the particular characteristics of this branch of spectroscopy, especially in relation to other phases of spectroscopy. Such an understanding will, in itself, promote an appreciation of how ultraviolet absorption spectroscopy can be used to solve specific problems of fat and oil chemistry and to which types of problems this particular tool is most likely to prove advantageous.

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Some Theoretical Considerations

One can immediately think of various branches of chemical spectroscopy—x-ray spectroscopy, ultraviolet spectroscopy, visible spectroscopy, infrared spectroscopy, and microwave spectroscopy. These terms are all really designations of various portions of the electromagnetic spectrum. In Figure 1 these various branches of spectroscopy have been listed with the respective regions of the electromagnetic spectrum with which each is related. Spectral radiation in any of these portions of the electromagnetic spectrum can be used for analytical purposes in several ways. The radiation emitted (emission or spectrochemical analysis and fluorescence spectroscopy—which may be considered a special or secondary type of emission) has found greatest utility as atomic spectra for the determination of the individual chemical elements. A study can be made of the absorption of any of these radiations by a specific sample under investigation (absorption spectroscopy). Or scattering or diffraction of each of these radiations can be utilized (Raman effect, x-ray diffraction spectroscopy, or visible reflection measurement of color).

As seen in Figure 1, spectroscopy is thus divided into 15 subdivisions. If one were to look hard enough and long enough, he could undoubtedly find applications of each of these 15 subdivisions to chemical spectroscopy but probably not, at the present time, to chemical spectroscopy of fats and oils. The subdivisions most frequently used in chemical spectroscopy are indicated by their commonly used names. From this figure one can obtain an exact definition of "ultraviolet absorption spectroscopy"—a study of the absorption, by any specific material, of that portion of the electromagnetic spectrum between about 2 and 400 mμ.

While this statement is an exact definition of ultraviolet absorption spectroscopy, it does not, in itself, add much to an understanding of the characteristics of this particular branch of spectroscopy to differentiate it from the other branches or to enable one to predict when it could be particularly useful to the solution of a specific problem in fat and oil chemistry.

When radiation is emitted, absorbed, or scattered by any material, the molecules of the material gain or lose energy. In spectroscopy we say that their energy level is changed. An absorption of radiation results in a gain of energy, an increase in energy level. Emission results in a loss of energy, a decrease in energy level. Scattering may involve either, or both. The origin of all spectra is explained by the Bohr theory involving the simple, fundamental relationship:

$$\triangle E = h\bar{\nu}$$

That is, change in energy content, $\triangle E$, (energy level) of any molecule by the absorption or emission of radiation is proportional to the frequency of the emitted or absorbed radiation, $\bar{\nu}$. The proportionality constant, h, is called Planck's constant. As the frequency, $\bar{\nu}$, can be related to wave number, ν', or to wavelength, λ, by the following relations:

$$\bar{\nu} = \nu' \times c = c/\lambda$$

in which c is the speed of light, the Bohr equation can be rewritten:

$$\triangle E = hc/\lambda$$

for those who feel more at home with wavelengths.

An important result of the quantum theory is that this energy which the molecule can gain or lose is quantized. It is beyond the scope of this lecture to delve into the rigorous mathematical relations of quantum theory or of wave mechanics. A familiarity with either is not a requisite for applying spectroscopy to the solution of chemical problems. Some knowledge of the results of such a treatment is however desirable.

The statement that changes in energy level are quantized merely means that the molecule cannot absorb or emit radiation continuously. Changes in energy, as related to these radiations by the Bohr equation, are restricted to a series of discrete values, bundles of $h\bar{\nu}$'s. Einstein showed that each discrete bundle of energy, $h\bar{\nu}$, was absorbed or emitted by a single molecule. The various size bundles that can be absorbed, or emitted by a specific molecule are dependent upon molecular structure. Hence studies of radiation absorbed or emitted must provide some clues to the structure of the molecule.

Experiments show that changes in energy during absorption and emission processes involve increases or decreases of one of three general orders of mag-

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nitude. The first of these is rather large, about 100 kilocalories per mole. A second is considerably less, about 1 kilocalorie per mole, and the third is still considerably less, about 0.01 kilocalorie per mole. From these energy values, by use of Bohr's equation, the ranges of radiations involved can be calculated:

$$100 \text{ kcal.} \simeq 35{,}000 \text{ cm}^{-1} = 0.3\ \mu \text{ or } 300 \text{ m}\mu \text{ (ultraviolet)}$$

$$1 \text{ kcal.} \simeq 350 \text{ cm}^{-1} = 30\ \mu \text{ (infrared)}$$

$$0.01 \text{ kcal.} \simeq 3.5 \text{ cm}^{-1} = 3000\ \mu \text{ or } 3 \text{ cm (microwave)}$$

From Figure 1 it can readily be seen that the first of these three energy changes is the one with which we are concerned in ultraviolet absorption spectroscopy; 300 mμ is in the ultraviolet portion of the electromagnetic spectrum.

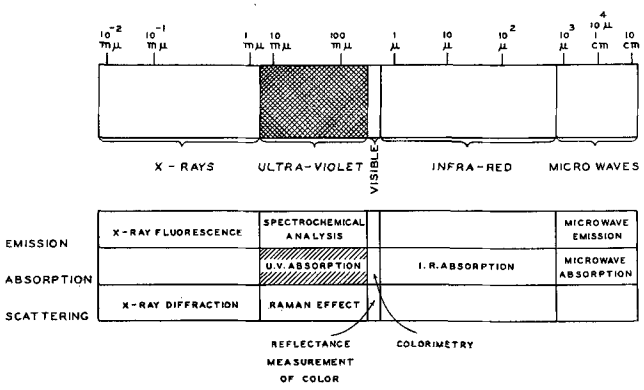

Fig. 1. Divisions of chemical spectroscopy.

If absorption were to consist of simply a change in the energy level of the molecule to another higher level, then the Bohr equation would be associated with a single frequency or wavelength, *i.e.*, the radiation would be monochromatic and the absorption would appear as a single line. This is the case of absorption of atoms. However the total energy of a molecule is the sum of four types:

$$E = E_{\text{Translational}} + E_{\text{Electronic}} + E_{\text{Vibrational}} + E_{\text{Rotational}}$$

As translational energy has no significant effect on spectra, it will not be considered further. Electronic energy is considerably greater than vibrational energy, and rotational energy is considerably less than vibrational energy. Figure 2 is a schematic method of illustrating energy levels and transitions of electrons from one energy level to another, called an "energy level diagram." It will be seen that transitions from one electronic level to another involve the greatest energy changes, the order of magnitude *ca.* 100 kcal. we encountered before. Vibrational transitions involve intermediate energy changes, *ca.* 1 kcal., and rotational transitions the least, *ca.* 0.01 kcal. Within certain restrictions of Pauli's exclusion principle and of the selection rules, the molecule will, in going from one electronic level, say the ground state, E_0, to a higher electronic level, say the first excited state, E_1, during the absorption of radiant energy, undergo simultaneously changes in vibrational energy and in rotational energy levels. As a result a series of several lines occurs which are very close together in the wavelength or frequency scale. When measured with the dispersion of most instruments used in chemical spectroscopy, these lines are not resolved from one another, and the absorption is measured over a portion of the electromagnetic spectrum. The portion in which absorption occurs is called an absorption band. Molecular absorption therefore results in band spectra, and ultraviolet absorption spectra, involving the relatively large energy changes, in the order of magnitude of 100 kcal. per mole, must arise from electronic energy level transitions. Thus ultraviolet absorption spectra are electronic band spectra. The intermediate values of energy changes arise from transitions between vibrational levels within a single electronic level and, as seen before, are of a magnitude associated with the infrared region. The smallest energy changes arise from the pure rotational transitions within a single vibrational level, and as their magnitude indicates, they are associated with microwave spectroscopy.

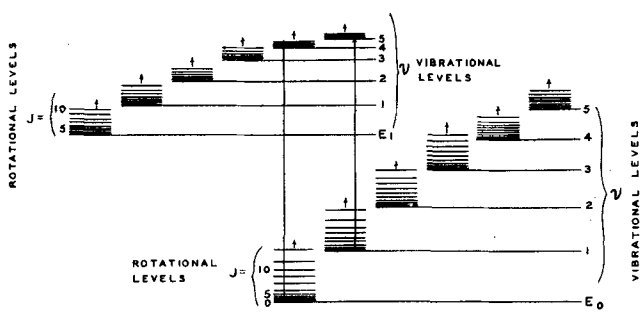

Fig. 2. Energy level table—Electronic transitions—Diatomic molecule.

In Figure 2 only two electronic energy levels, E_0 and E_1, are shown. The diagram illustrates that: a) vibrational levels are more closely spaced than electronic levels, b) rotational levels are even more closely spaced than vibrational levels, c) both vibrational and rotational level spacing differ for different electronic levels, d) vibrational level spacing decreases as the vibrational level number, v, increases and e) rotational level spacing increases as the rotational level number, J, increases. It can readily be seen that the number of energy level changes involving electronic, vibrational, and rotational levels is very great. While selection rules somewhat decrease the number of transitions permitted, the number of bands even in the spectra of simple molecules is very numerous and the electronic spectra are very complex.

Experimental Measurements and Methods

The upper wavelength limit of the ultraviolet portion of the electromagnetic spectrum is where it runs into the visible region, *i.e.*, the lowest wavelength at which the eye can be used as a detector. This position depends somewhat on the eyes of the individual, but it is usually considered about 400 mμ. The lower limit of ultraviolet spectra is the x-ray region about 20A. Below this wavelength absorption arises mainly from transitions of inner-orbital electrons, *i.e.*, x-ray spectra. However a limitation of instrumentation places the lower limit of practical ultraviolet absorption at about 200 mμ. Several factors contribute to this limitation: a) quartz, the usual material for optics in the ultraviolet region, begins to absorb, b) the detectors, *i.e.*, photoelectric cells, become less

sensitive, c) the light sources, *i.e.*, the hydrogen discharge lamp, become weaker, and d) air soon begins to absorb radiation and a complete vacuum is required. While vacuum spectrophotometers, operating with gratings to avoid the absorption of quartz optics and with special types of detectors sensitive to short wavelength ultraviolet radiation, have been designed to permit measurements particularly in the region of ethylenic absorption about 170 to 190 mμ, commercially available instruments have usually been restricted to the region above 200 mμ. The usable ultraviolet absorption region for chemical spectroscopy may be considered to be 200 to 400 mμ. (It may be noted however that Rusoff, Platt, Klevens, and Burr (45) have reported the ultraviolet spectra of several fatty acids and related compounds down to 170 mμ.)

If the absorption of any material is measured as a function of the wavelength throughout this region, we would find that the resulting spectrum would be one of two general classes, noncharacteristic, with smooth absorption generally increasing gradually toward the shorter wavelengths, or selective absorption exhibiting characteristic maxima and minima. Noncharacteristic absorption, illustrated in Figure 3-a and c, arises from end-absorption of intense bands usually due to single C=C, ethylenic, or to COOH, carboxyl groups with absorption maxima between about 175 and 185 mμ. While the magnitude of this absorption has been suggested as an indication of the presence and amount of these groups, in general this type of absorption is of no importance to chemical spectroscopic studies. Thus we are concerned with the determination of which types of compounds give rise to selective absorption, illustrated in Figure 3-b and d. The appearance of an absorption band must depend ultimately on the electronic energy levels of the molecule as it is a transition between two such energy levels which gives rise to the radiation, according to the Bohr equation. With instruments of very high resolution the various lines of the band spectra have been analyzed for a few very simple molecules, and from these data the entire energy level diagrams of the compound have been computed. However the complexity of electronic spectra have prevented much progress in this manner for polyatomic molecules. The molecular orbital method has had considerable success as an approximation for such a treatment. The molecular orbital method is beyond the scope of this lecture, but in principle it is based on a treatment of only the π electrons of the unsaturated molecule. Thus in benzene only the six π electrons (or unsaturated electrons) are considered. The remainder of the benzene molecule is considered as a framework over which these electrons are distributed and free to move. An excited benzene molecule corresponds to a different distribution of the six π electrons.

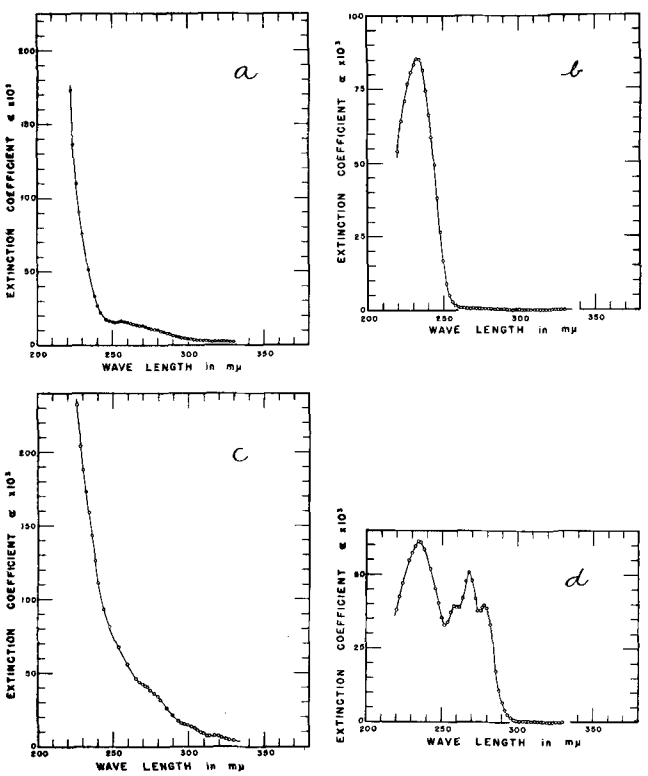

FIG. 3. Ultraviolet absorption spectra: (a) and (c) smooth absorption of linoleic and linolenic acids, (b) and (d) characteristic absorption of alkali-isomerized linoleic and linolenic acids.

From this discussion it is apparent that selective absorption in the near ultraviolet region may be expected only for unsaturated molecules. But experiment shows that not all unsaturated molecules give rise to characteristic bands in this region. The absorption arising from single unsaturated groups is usually very weak and often will not appear at all except as the end-absorption from bands below the 200 mμ limitation previously discussed. This is illustrated by Figure 3-a and c, the spectra of very pure linoleic and linolenic acids. These molecules have isolated C=C groups and a COOH group and exhibits intense absorption with maxima about 170 to 180 mμ (45). The only absorption observed above 200 mμ is the end-absorption from these intense bands, decreasing rapidly toward the longer wavelengths.

However conjugation of a single unsaturated linkage with a second unsaturated group gives rise to intense absorption bands throughout the region 200 to 400 mμ. Conjugation of two ethylenic linkages, forming a conjugated dienoic system, C=C—C=C, gives rise to an absorption band of considerable intensity in the region about 230 mμ. The exact position of maximum of this band can be calculated by means of Woodward's rule. This rule, applied to acyclic conjugated dienes, assigns a value of 5 mμ to be added to the position of maximum absorption for the simplest conjugated diene (butadiene, *ca.* 217 mμ in isooctane solution) for each alkyl substituent linked to the diene chromophore. Extension of the conjugated system to a trienonic conjugation, C=C—C=C—C=C, results in a shift in the absorption to about 268 mμ while a tetraenoic system absorbs at about 315 mμ, pentaenoic at about 346 mμ, and hexaenoic at about 374 mμ. Continuation of the conjugated system soon brings the absorption into the visible region, and colored compounds are obtained. We come into Hewitt's rule which has been known since 1907 and which states that the longer the conjugated unsaturated system, the deeper the color.

While conjugations of other various combinations of single unsaturated linkages have limited usefulnes, the specific conjugations of two or more ethylenic linkages are responsible for the great majority of the successful applications of ultraviolet absorption

spectroscopy in fat and oil chemistry. It can readily be seen how an observation of the ultraviolet spectrum of an unknown fat or oil can answer such questions as: a) does it contain any unsaturated conjugated material? b) if conjugated substances are present, what is the order of conjugation? c) if there is evidence of diene conjugation, what does Woodward's rule say about the nature of the substituents? The particular characteristics of the ring triene conjugation of aromatic compounds can soon be recognized and the question—is the material aromatic or aliphatic?—can readily be answered. Conjugation in nonaromatic rings gives rise to characteristic absorption bands which can be recognized by extensions of Woodward's rule. It should be readily apparent that a tool which can furnish information to answer these types of questions readily, simply, and rapidly and usually without destroying the sample cannot fail to find useful applications in the field of fat and oil chemistry.

Two laws which are important to the use of absorption spectra as a tool of quantitative analysis are those of Bouguer and of Beer. Bouguer's law states that the proportion of radiation absorbed by a transparent medium is independent of the intensity of the incident radiation and that each successive unit layer of the medium absorbs an equal fraction of the radiation passing through it. Mathematically expressed, where P is the transmitted radiant power, b the length of absorbing path, and k a proportionality constant:

$$-dP/db = kP$$

The mathematical expression can be integrated over the thickness of the medium, b:

$$\text{Log}_{10} P_o/P = kb$$

where the logarithm of the ratio P_o/P, the transmitted radiant power before to that after passing through thickness b, is known as the absorbancy, A. Thus

$$k = A/b$$

Beer's law states that the light absorption is proportional to the number of molecules of absorbing substances through which the light passes. Or expressed mathematically:

$$-dP/dc = k'P;\ \text{Log}_{10} P_o/P = k'c;\ k' = A/c$$

The two laws can be combined into a single expression:

$$a = A/bc$$

Alpha is a constant characteristic of the absorbing medium and is known as the absorptivity. For a pure compound a, when measured with an instrument which permits sufficient resolution, is a characteristic constant of the compound, as characteristic as is its melting point, boiling point, index of refraction, etc. If the absorbance of a material, A, is measured in a cell b cm. long, the concentration of the absorbing compound, in grams per liter, can be calculated if a, the characteristic constant for this absorbing compound, is known, by means of the expression:

$$c = A/ab$$

Applications to Problems in Fat and Oil Chemistry

As a first illustration of an actual use of ultraviolet absorption spectroscopy in fats and oils, the very simple determination of α-eleostearic acid in fresh α-tung oil has been selected (37). This particular analysis has been selected because its very simplicity affords an excellent opportunity to illustrate the method of spectrophotometry for quantitative determinations. Fresh, or so-called α, tung oil owes its particular properties as a drying oil to the presence of large quantities (ca. 80%) of α-eleostearic acid (9-*cis*, 11-*trans*, 13-*trans*-octadecatrienoic acid). This is a naturally conjugated trienoic acid and, as expected, exhibits intense absorption with a maximum at 271.5 mμ (Figure 4-A). Estimations of the quality of tung

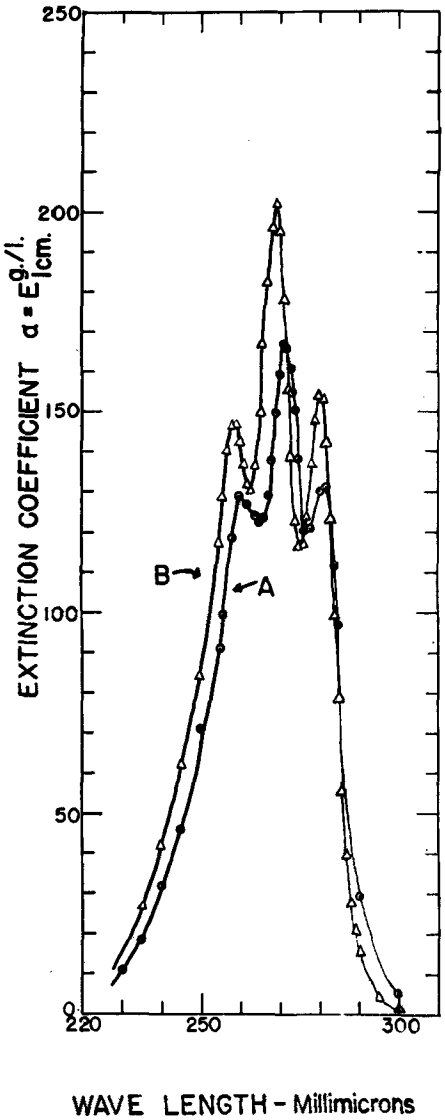

FIG. 4. Ultraviolet absorption of eleostearic acid: (a) α-isomer, (b) β-isomer.

oil have been made by a long, tedious, and not entirely satisfactory determination of eleostearic acid by empirical calculations from maleic anhydride addition reactions. If a few drops of the oil are accurately weighed into a small volumetric flask, dissolved in cyclohexane, and brought to volume with the same solvent, a single spectrophotometric measurement at 271.5 mμ will provide the data required to calculate the concentration of the eleostearic acid in the tung oil sample from the Bouguer-Beer equation: [The absorptivity of α-eleostearic acid at its maximum at

271.5 mμ has been established for cyclohexane solutions as 168.6 (38).]

$$C = A/(168.6 \times b)$$

The complete analysis can be made in a few minutes as compared to the day-long maleic anhydride addition method. No other component of fresh tung oil contributes to the absorption at 271.5 mμ, and no corrections for interferences are required. Thus we have an excellent illustration of a direct, simple spectrophotometric determination.

As tung oil ages, its viscosity may increase, and it may, under some conditions, become solid. The oil is then known as β-tung oil. This change is due in large measure to an isomerization of α-eleostearic acid to the β-isomer. Work of two groups, in particular (7, 42), using infrared absorption spectroscopy, have established the molecular configuration of these two acids as 9-*cis*, 11-*trans*, 13-*trans*-octadecatrienoic and 9-*trans*, 11-*trans*, 13-*trans*-octadecatrienoic acids, respectively. Their ultraviolet absorption is similar but not identical (Figure 4). Alpha-eleostearic acid exhibits a maximum in cyclohexane solution at 271.5 mμ, $a = 168.6$ (38). Beta-eleostearic acid exhibits a maximum at 269.0 mμ, $a = 202.4$ (38). In any tung oil the relative proportions of these two components can be determined by use of the technique known as spectrophotometric multicomponent analysis. The total absorption of a tung oil sample at 271.5 mμ, the maximum for the α-eleostearic acid isomer, will be due to α-eleostearic acid and to the contribution of the β-isomer at this wavelength. For a pure sample of β-eleostearic acid this contribution at 271.5 mμ is $a = 178.1$ (38). Similarly at 269.0 mμ, the maximum position for absorption of β-eleostearic acid, the total absorption of any tung oil will be the absorption due to the β-eleostearic acid plus that contributed by the α-eleostearic acid at this wavelength. Pure α-eleostearic acid contributes, $a = 149.5$, at 269.0 mμ. Thus:

> Total measured absorptivity of any tung oil sample at 271.5 mμ = (168.6 × % α-acid + 178.1 × % β-acid)/100.

Similarly:

> Total measured absorptivity of any tung oil sample at 269.0 mμ = (149.5 × % α-acid + 202.4 × % β-acid)/100.

Simultaneous solution of the two equations and spectrophotometric measurement and calculations of the two absorptivities at the two wavelengths yield the percentage of both α- and β-eleostearic acids in the particular sample.

From Figure 4 it can be seen that the absorptivity curves cross at 276.5 mμ. At this point the total absorption is independent of the ratio of α- and β-isomers. Such an absorption position is called an "isosbestic point." It can be used as a direct determination of total eleostearic acid to check the sum of the α- plus β-acids obtained from the simultaneous equations. The absorptivity at this isosbestic point has been established as 122.5 (38).

$$\text{Total eleostearic acid} = A/(122.5 \times b)$$

Thus we have an illustration of multicomponent analysis applied to a drying oil. The general method can be extended, with increasing mathematical complexity, to three, four, five or more components, all of which must exhibit selective absorption with well resolved maxima. Ultraviolet spectroscopy has permitted not only a much more rapid determination of eleostearic acid than is possible by chemical methods, but it affords as simple a means for determination of the relative amounts of the two isomers, an analysis which cannot be made by chemical means, and one of considerable importance in cases where solidification of a supply of oil is of some concern, as in a tank car shipment.

Application to the Determination of Nonconjugated Polyunsaturated Fatty Acids

The most profitable application of ultraviolet absorption spectroscopy to fat and oil chemistry has been the quantitative determination of nonconjugated polyunsaturated acids in fats, oils, triglycerides, esters, and related substances and various derivatives. These analyses are also based on the principle of multicomponent analysis. However, as polyunsaturated acids, *i.e.*, linoleic, linolenic, and arachidonic acids, etc., in most oils and fats are nonconjugated acids, their analysis illustrates another fundamental technique of ultraviolet spectroscopy. This technique can perhaps best be introduced by a comparison with a similar principle in colorimetric measurements, using visible absorption spectroscopy. Many colorless compounds (which therefore do not exhibit selective absorption in the visible portions of the spectra) are determined by means of visual colorimetry. The technique involves merely the production of color by some suitable chemical reaction, measurement of the color, and calculation of the amount of the sought-for constituent which would produce the measured color. Both production and measurement of the color are performed as a careful, specific, analytical technique. Similarly ultraviolet absorption can be produced by careful, specific, reproducible means to create conjugation. Spectrophotometric measurement of this produced conjugation and calculations, which includes consideration of the amount of conjugation produced by the selected procedure, provides a quantitative method of analysis.

Dan and Moore (13) and Moore (29) showed that linoleic and linolenic acids are respectively isomerized to conjugated dienoic and to mixtures of conjugated dienoic and trienoic acids by heating with alcoholic potassium hydroxide. Kass, Miller, and Burr (23) showed that this isomerization is effected much more rapidly at higher temperatures, *i.e.*, with the use of a solution of alcoholic potassium hydroxide and ethylene glycol at 180°C. Mitchell, Kraybill, and Zscheile (28) used this isomerization procedure to establish a quantitative spectrophotometric method for the direct determination of the linoleic and linolenic acid content of a fat.

The extreme popularity of the method is evidenced by the great number of publications proposing applications, tests, and modifications of original method. Now, about 12 years after proposal of the original method, it may be said to have undergone a period of considerable evolution. The method was first proposed for the determination of linoleic and linolenic acids. It was shortly extended to include arachidonic acid by Brice and his coworkers (8, 10), using absorptivities proposed by Beadle and Kraybill (5). Later it was extended to include pentaenoic acids by Herb and Riemenschneider (16), and Hammond and

Lundberg (14) have recently suggested the inclusion of hexaenoic acids. Brice and his coworkers (8, 10) introduced mathematical equations to correct for "background" absorption when applying the method to very small quantities of linoleic, linolenic, and arachidonic acids in animal fats and tallows. These corrections, while of considerable value when measuring small quantities, 1% or less, of the constituent acids, are not only unnecessary expenditures of effort and time, but are actually undesirable in the analysis of most vegetable and drying oils, where acid concentration is of the order of 20 to 50%. O'Connor, Heinzelman, and Dollear (36), using the method to determine soybean oil admixed with cottonseed oil by measuring the trienoic conjugation arising from the linolenic acid content of the former oil only, proposed complete nitrogen blanketing both during the isomerization and during the preparation of the reagent in order to avoid oxidation of the readily oxidizable components. Brice *et al.* (9) used pure *cis*-linoleic, linolenic, and arachidonic acids prepared by Riemenschneider *et al.* (44) and by Herb *et al.* (18) to redetermine absorptivities for these natural isomers, greatly improving the accuracy of the procedure.

A factor which caused some difficulties in the spectrophotometric method is the presence of conjugated material found in some oils before alkali isomerization. These preformed conjugated acids were originally considered to be caused by isomerization of the corresponding nonconjugated acid during storage or handling of the oil. O'Connor, Heinzelman, Caravella, and Bauer (35) demonstrated the readily oxidizable properties of both linoleic and linolenic acids to yield conjugation of the next higher order. Swift, O'Connor, Brown, and Dollear (49), reporting on the oxidation products of cottonseed, showed that smooth broad-banded absorption in the region of triene conjugation arises from conjugated decadienals. Swain and Brice (47) made a very comprehensive study of the formation of these preformed conjugated constituents. As a result of these studies it is now well established that preformed conjugation is more likely a measure of the autoxidation of an oil rather than evidence for conjugated fatty acids.

Several discrepancies were reported in measurements of partially hydrogenated fats and oils by the alkali isomerization-ultraviolet spectrophotometric method. Work of Riemenschneider, Nichols, and Herb (32, 33, 44) showed that the rate of isomerization and the magnitude of the absorptivities obtained vary for various *cis,cis-* and *cis,trans-* and *trans,trans*-acids. Therefore the accuracy of the method based on *cis*-acids only will not be satisfactory when samples, such as partially hydrogenated materials, which can be shown by infrared absorption spectroscopy to contain mixtures of *cis-* and *trans*-acids, are analyzed. The spectrophotometric method is applicable only to naturally occurring products, *i.e.*, products containing only *cis*-acids. Brice *et al.* (9) showed the excellent agreement which can be obtained by the spectrophotometric method when analyzing all *cis*-containing products. O'Connor *et al.* (41) made a detailed comparison with the chemical methods for the analysis of a number of cottonseed oils and also showed good agreement. Baldwin and Longenecker (4) tested the method by the analysis of known mixtures of purified methyl esters, and Baldwin and Daubert (3) tested the method with the analysis of synthetic glycerides.

The present American Oil Chemists' Society Tentative Method Cd 7-48 (1) for the determination of polyunsaturated acids is a considerably revised modification of the earlier Mitchell, Kraybill, and Zscheile method, representing the results of exhaustive investigations by the Spectroscopy Committee for a number of years on scores of proposed changes. The most recent report of the committee (43) recommends a revised method which will permit the determination of linoleic, linolenic, arachidonic, and pentaenoic acids and which: a) incorporates the "background" corrections of Brice *et al.* (8, 10) only if small traces of the acids are to be determined, b) employs the nitrogen-blanketing protection method of O'Connor *et al.* (36), c) uses the constants for natural acids proposed by Brice *et al.* (9), and d) permits several simplifications when the materials being analyzed do not contain all of the various polyunsaturated acids. Cottonseed oil, with only diene unsaturation, is analyzed for linoleic acid by a single measurement in the diene region at 233 mμ (41). The Spectroscopy Committee has repeatedly tested and confirmed the reliability of the method in collaborative studies in several laboratories and has concluded that within its scope reasonably reproducible results can be obtained by experienced operators (43).

At the present time the two most serious limitations in the scope of the method are inability to analyze accurately materials which contain *trans*-acids, and difficulties in the analysis of samples which contain large quantities of preformed conjugation, *i.e.*, tung oil with 80% triene conjugated eleostearic acid. A method for the determination of linoleic acid in tung oil and similar samples has recently been proposed by O'Connor *et al.* (39) and is now being tested by the Spectroscopy Committee. Development of accurate methods to determine the proportion of *cis-* and *trans*-acids by means of infrared absorption spectra may provide a method whereby such mixtures can be analyzed by the alkali isomerization-ultraviolet absorption procedure.

Several interesting applications of the alkali isomerization-ultraviolet absorption spectrophotometric method have been reported, as the differentiation of lard from hydrogenated vegetable oils by means of the tetraenoic absorption appearing in the spectra after alkali isomerization of the arachidonic acid in the lard, reported by Beadle, Kraybill, and Stricker (6). Space will not permit description of more of these. However the introduction of a microtechnique by Herb and Riemenschneider (17), which has proven particularly useful in medical, biochemical, and other applications where sample size is a serious problem, should not be overlooked.

Other Examples of the Use of Ultraviolet Absorption Spectroscopy in Fat and Oil Chemistry

Considerable detail has been given to the discussion of the alkali isomerization-ultraviolet absorption spectrophotometric method for the quantitative determination of fatty acids. This proportion of space is commensurate with the relative amount of attention and importance that accrues to this one method of analysis. There are however several other applications of ultraviolet absorption spectroscopy which, within their field, are of considerable importance and value.

Only a few of these can be mentioned most briefly here.

Vitamin A exhibits a spectrum with a characteristic maximum at 325 mμ (Figure 5). This absorption can be used in a direct ultraviolet spectrophotometric quantitative determination of the vitamin. Details of the specific procedure are given in the Association of Vitamin Chemists' "Methods of Vitamin Assay" (2). Luckmann, Melnick, and coworkers in a series of four papers (24, 25, 27) reported exhaustive studies of this method for the determination of vitamin A in fortified fats, particularly margarine, and in various

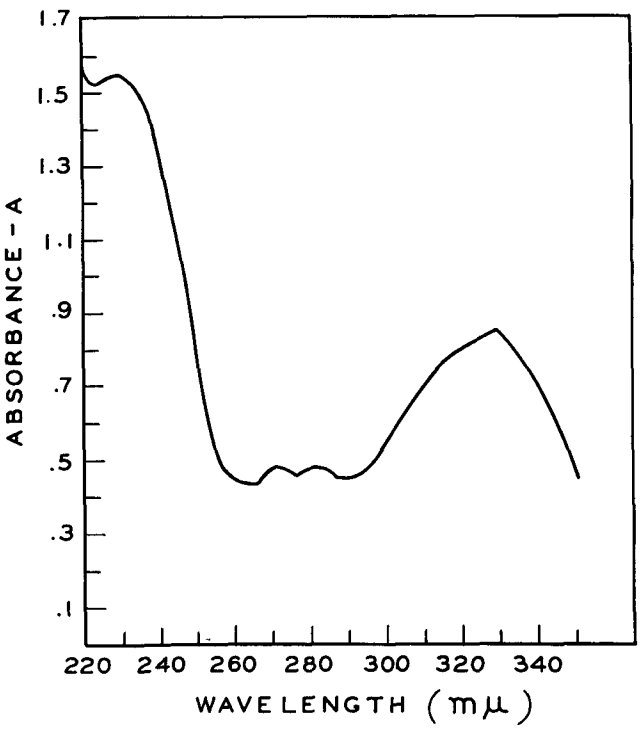

FIG. 5. Ultraviolet absorption of vitamin A.

vitamin A concentrates. They concluded that ". . . the spectrophotometric method is the most reliable procedure for assaying margarine for vitamin A following fortification with quality vitamin A concentrates. It is far more precise than the biological assay and equally as specific for vitamin A . . ." If the unfortified material is available, the determination can best be made by measuring both the fortified and nonfortified sample at 325 mμ. Vitamin A in U.S.P. units can then be obtained from the relation:

$$\text{U.S.P. units of vitamin A per gram} = (a'-a) \times 5{,}700 \times 1/0.3$$

where a' and a are absorptivities at 325 mμ for the fortified and nonfortified samples, respectively, 5,700 is a factor for conversion from spectrophotometric to gravimetric units, and 0.3 is a factor to convert grams to U.S.P. units. If the nonfortified sample is not available a correction for absorption at 325 mμ, not due to vitamin A, can be made by a mathematical method (30). Murray, O'Connor, Suarez C., and Bickford recently applied the technique to show that vitamin A was reasonably stable in sesame oil (31).

Of the various nonconjugated groups which exhibit low magnitude selective absorption in the ultraviolet region of the spectrum, the carbonyl grouping, C=O, has been the most useful in application to fats and oils. The C=O group of aldehydes or ketones, but not of acids or esters, gives rise to low broad characteristic bands between about 265 and 285 mμ. When this C=O group is conjugated to the ethylenic C=C linkage, conjugated absorption in the diene, triene, tetraene, etc., regions is produced, which is of considerably higher magnitude than that of the nonconjugated group. In a series of six papers entitled "Spectrophotometric Studies of the Oxidation of Fats," Holman, Lundberg, Lauer, and Burr (19, 20, 21) report results of an exhaustive study of the changes in ultraviolet absorption during the oxidation of fatty acids, esters, and various fats. They tabulated correlations between the observed changes in the selective absorption and the oxidation reactions and concluded that increase in absorption during oxidation is not due to peroxide formation but probably arises, in part at least, from conjugated unsaturated systems containing carbonyl groups or from conjugated polyenes formed by enolization of these systems.

As mentioned earlier, Swain and Brice (47) made several studies of the changes in ultraviolet absorption during the heating of unoxidized fatty acids and fats in neutral ethylene glycol and concluded that increase in conjugated absorption was due to oxidation effects, not to isomerization of nonconjugated to conjugated systems. Swift *et al.* (48, 49) showed that the oxidation products of methyl oleate and of cottonseed oil included α,β-unsaturated aldehydes and dienals which absorb selectively in the region of diene and triene conjugated absorption. They attributed the absorption to conjugation of the carbonyl and ethylenic linkages.

Japanese workers have reported several studies of the oxidation of fatty acids by means of ultraviolet absorption spectrophotometry. Toyama (50) has recently summarized this work, describing the course of oxidation of several pure acids when oxidized with different reagents. The utility of ultraviolet absorption spectra of the acids before and after alkali isomerization, and of their oxidation and scission products in interpreting the results obtained, is repeatedly emphasized.

The use of ultraviolet absorption spectra to follow the course of oxidation reactions has been employed in drying oil studies. The mechanism of heat-bodying, rate of heat polymerization, and changes in spectral properties during the course of air- or of oxygen-blowing of oils and during hydrogenation of oils have been reported. A review of these applications by Kass will be found in Mattiello's "Protective and Decorative Coatings" (22). "Spectroscopic Changes in Fats During Rancidification" is the title of a paper by Lundberg, Holman, and Burr (26). They considered their studies preliminary in nature but as clearly emphasizing that there is promise of much knowledge to be gained concerning the course and mechanism of the autoxidation of fats, at least in the early stages, through spectroscopic studies. Hendrickson, Cox, and Konen later reported on "Some Applications of Ultraviolet Spectrophotometry in Drying Oil Research" (15). The drying or autoxidation of an oil film was shown to proceed as a three-stage reaction: a) a short, initial stage, characterized by an accumulation of conjugated diene and

triene structures with some evidence of increase in α-keto groupings capable of enolizing to give absorption maxima characteristic of triene and tetraene conjugated systems, b) a slow decrease in the measurable conjugated diene and triene structures, and c) a third and final deterioration stage. The principle that the quantity of light absorbed by an oil film in the region 320 to 400 mμ is indicative of the durability of the film upon exposure to accelerated weathering conditions was verified. Ultraviolet spectrophotometry, it was concluded, is a valuable tool for investigating some of the chemical changes which occur as a vegetable oil film dries and ages.

Changes in absorption spectra during refining, bleaching, and deodorization of oils have been studied. The significant changes in spectral properties for several types of the more common vegetable and drying oils have recently been described and interpreted by O'Connor and coworkers (34, 40).

In Figures 6 and 7 the ultraviolet absorption spectra of (A) sesamin, (B) sesamolin, (C) sesamol, and (D) a crude sesame oil are shown. Sesamin is a constituent of sesame oil which has received considerable interest as a synergist for the pyrethrins. Sesamolin and sesamol are antioxidants to which sesame oil owes its remarkable stability. Budowski, O'Connor, and Field (11, 12) used ultraviolet absorption spectros-

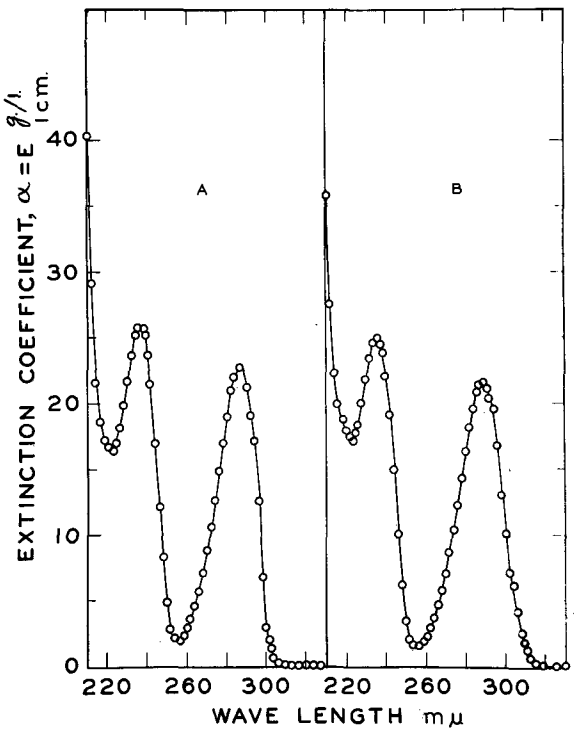

Fig. 6. Ultraviolet absorption of (A) sesamin, (B) sesamolin.

copy in connection with the well-known Villavecchia color reaction to determine these three constituents simultaneously in sesame oils. Suarez C., O'Connor, Field, and Bickford modified the method and extended it to various sesamin concentrates (46).

Conclusions

In a single lecture on a subject as big as ultraviolet absorption spectroscopy as applied to fats and oils, the contributions of many, many workers cannot even be mentioned. Most of the techniques and much of the mass of useful data which have been accumulated represent contributions from scores of research workers. In this lecture only a few have been cited as examples of what ultraviolet absorption spectroscopy can do—what it is doing for the fat and oil chemist.

As one hears more about newer techniques in spectroscopy, infrared absorption, microwave spectroscopy, nuclear magnetic resonance spectroscopy, etc., he is likely to think of ultraviolet absorption as an older technique which has probably made its contribution and is now to be relegated to "classical

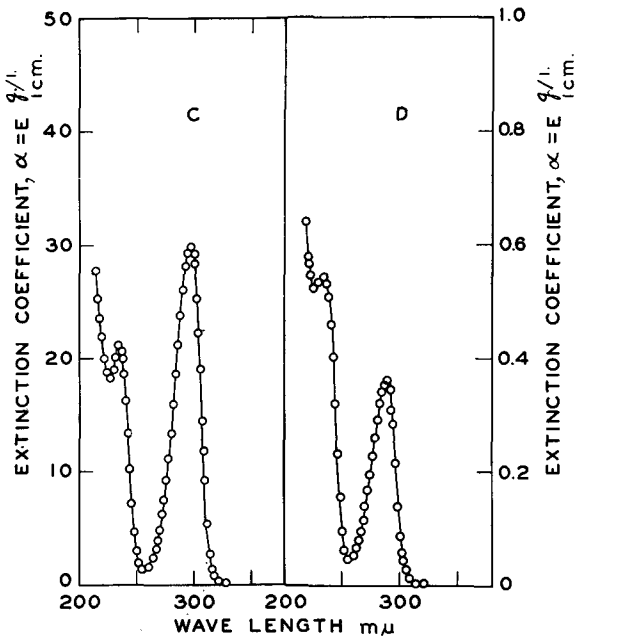

Fig. 7. Ultraviolet absorption of (C) sesamol, (D) crude sesame oil.

methods." Nothing could be further from the actual picture today. As newer techniques open up a greater understanding of the chemistry of fats and oils, of their constitution, of the mechanisms of their chemical reactions, ultraviolet absorption spectroscopy will be called upon to make on ever-increasing contribution. Newer techniques to obtain pure components, such as improved chromatography, countercurrent distribution separations, etc., will also make greater and greater demands upon the use of ultraviolet absorption spectra. As long as fatty acid chemistry deals with constituents which either contain conjugated unsaturation, or unsaturated linkages which can be conjugated, either accidentally or by design, ultraviolet absorption spectroscopy will remain its most valuable tool.

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Infrared Absorption Spectra

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A RECENT REVIEW of the applications of infrared absorption spectrophotometry to problems in fat and oil chemistry (50) revealed that during the four-year period from 1950 through 1954 more than 100 papers dealing with this subject had appeared in the technical literature. The report showed that application of infrared absorption techniques is widespread, reaching just about all phases of the fat and oil industry.

The purpose of this paper is to discuss the characteristics of infrared absorption, to show how this branch of spectroscopy can be of considerable use in fat and oil chemistry, and to illustrate with specific examples some of its successful applications.

Figure 1, listing the various branches of spectroscopy in 15 subdivisions based on radiations in various portions of the electromagnetic spectrum and the use to which these radiations are put, provides a definition of "infrared absorption spectroscopy"—a study of the absorption by any specific material, of radiation in that portion of the electromagnetic spectrum between about 1 and 100 μ. A consideration of the energy relations of radiations in this wavelength range of the electromagnetic spectrum with the aid of the fundamental equation of Bohr,

$$\triangle E = h\,c/\lambda$$

(E = energy, h = Planck's constant, c = speed of light, and λ = wavelength), shows that they are related to energy level changes of about 1 kcal. (1 kcal. is equivalent to 30 μ.) Thus infrared absorption spectroscopy is intermediate in the three orders of magnitude of energy level changes, *ca.* 100 kcal., 1 kcal., and 0.01 kcal. Energy level changes of this intermediate value are changes in vibrational levels (Figure 2). Hence infrared absorption spectroscopy is vibrational spectra. Figure 2 illustrates that vibrational changes do not occur without accompanying changes in rotational levels. Hence absorption or emission of molecules in the infrared will, like electronic spectra, appear as bands consisting of many lines so close together in wavelength that, with the commercial spectrophotometers used in chemical spectroscopy, they will not be resolved. Infrared absorption spectra is thus vibrational (or strictly vibrational-rotational) band spectra involving intermediate values

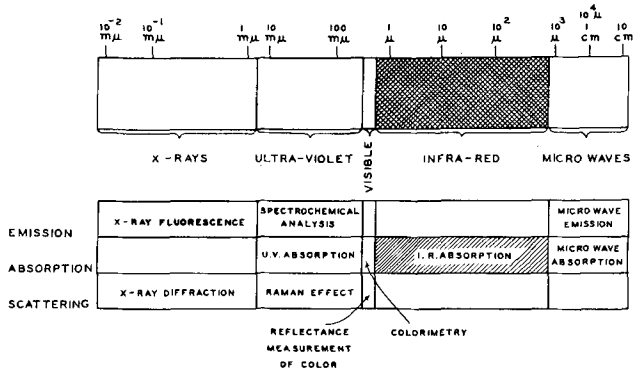

FIG. 1. Divisions of Chemical Spectroscopy.

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